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The Effect of Prostaglandins and Aspirin on Insulin Release from Isolated Normal Rat Islets

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THE EFFECT OF PROSTAGLANDINS AND ASPIRIN ON INSULIN RELEASE FROM ISOLATED NORMAL RAT ISLETS

by

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INTRODUCTION

The prostaglandins have gained importance in many aspects of physiology since their discovery by von Euler in 1935. They are found in almost every tissue and influence a variety of cellular responses including hormone release. Because of these effects, researchers searching for potential insulin secretagogues for possible application to treatment of diabetes have been examining the prostaglandins for influences they may have on insulin secretion.

The prostaglandin E family, especially Prostaglandin E_2 (PGE_2), has been studied by several investigators for the effect it may have on insulin release. These experiments have produced mixed results. Perhaps the strongest evidence to support the enhancement of rat pancreatic insulin release by PGE_2 *in vitro* was obtained by Johnson[1], who found an almost two-fold increase in PGE_2-stimulated insulin release in the presence of high glucose as compared with control insulin levels from glucose stimulation alone. Pek[2] and co-workers also noticed an increase in insulin release within a minute following perfusion of rat pancreas. On the other hand, several groups have shown inhibition of insulin release with PGE_2 administration. Sacca[3] found a significant decrease in plasma insulin concentration after giving rats intravenous prostaglandin E_1 or E_2 infusion. Another *in vivo* inhibitory effect was shown by Robertson[4] when he infused dogs with PGE_2 followed by glucose

stimulation and measured the degree of insulin response to be lower than in controls stimulated with glucose alone. Similar inhibition has been demonstrated using PGE_2 in rats.[5] This effect, however, may not be a direct one, because the results of Rossini et al.[6] suggest that the increase in insulin release seen in other experiments in vivo is due to a regional increase in pancreatic blood flow. They obtained no change in insulin release after isolated islet exposure to PGE_2. The experiments performed in the present study were undertaken to help clarify the role of PGE_2 on insulin release.

Since the time-course for insulin release appears to be biphasic, it would be of interest to examine when PGE_2 exerts its effect. The primary peak of insulin release occurs within the first few minutes after glucose administration and is followed by a decrease in insulin release. A second, more gradual rise is seen within thirty minutes after islet stimulation. Grodsky[7] used humans to demonstrate a biphasic response to glucose infusion with a primary peak occurring after three to four minutes exposure to glucose. Ashby and Speake[8] also clearly showed a primary peak of insulin release from isolated rat islets within two to four minutes of 16 mM glucose stimulation. Soeldner and Slone[9] performed a pulsed glucose administration test and still observed quick release of insulin within the first few minutes. Assuming, then, that the primary peak of insulin release has been reached by four minutes

after islet exposure to a stimulus (i.e. 300 mg percent glucose), an experiment was performed to determine if PGE_2 exerts its effect on the islets during the primary phase of insulin release in medium containing PGE_2 and 300 mg percent (%) glucose.

A compound similar to PGE_2, called Interoxaphenylene-PGE_1 (IOP-PGE_1) or U51095, has recently been synthesized and tested for potency in hematologic studies. Nishizawa[10] found U51095 to be approximately thirty times more potent than PGE_2 in platelet aggregation studies. The effect of U51095 on insulin secretion would determine whether or not this synthetic compound behaves similarly to PGE_2 in islet studies.

The prostaglandin synthetase complex is present in those tissues containing prostaglandins. As a consequence, the prostaglandins are synthesized within the tissues. It is possible to block the formation of prostaglandins by interfering with this enzyme system. Aspirin is one of several non-steroidal inhibitors of prostaglandin synthesis *in vivo* and *in vitro*. Smith and Willis[11] found that aspirin selectively inhibited the production of prostaglandins *in vitro*. Also, Smith and Lands[12] were able to demonstrate *in vivo* prostaglandin inhibition when they used cell-free homogenates of guinea pig lung exposed to aspirin. Karim[13] has listed $[I]_{50}$ values for inhibition of prostaglandin synthesis by aspirin and indomethacin. He stated that the values vary over three orders of magnitude for the two drugs in their effectiveness on certain tissues.

For insulin, the range is from 10.7 uM aspirin in guinea pig chopped lung to 15,000 uM aspirin in bovine lyopholized seminal vesicle microsomes. Some of the variation may be due to experimental technique.

The mechanism of action for aspirin on the prostaglandin synthetase system is beginning to be elucidated. Jafari[14] studied the effect of aspirin on prostaglandin synthesis in human platelets. It was their finding that the inhibitory action of aspirin occurs at the lipid peroxidation step in the synthetase pathway. They believe that the aspirin competes with substrate for binding to the cyclo-oxygenase enzyme and acetylates this enzyme competitively, thus inhibiting the enzyme. Rome[15] and co-workers confirmed Jafari's results, showing that aspirin acetylates the fatty acid oxygenase. This acetylation attenuates further prostaglandin production.[16] Studies on the effect of aspirin on insulin release were undertaken to determine whether aspirin could alter the effect of the prostaglandins and to determine whether aspirin exerts an effect of its own on the islets.

MATERIALS AND METHODS

Islets of Langerhans were isolated from young Upjohn strain rats using a collagenase digestion technique. The islets were then grouped and placed in pre-incubation medium containing non-stimulatory glucose levels for the stabilization of the islets and measurement of basal insulin levels. Finally, the islets were incubated in a specific experimental medium for a designated period of time in order to detect any change in insulin release. After removal of the islets, the medium was measured for insulin levels by radio-immunoassay.

Preparation of Solutions and Materials

Hanks Balanced Salt Solution (HBSS, Gibco) containing 50 mg percent (%) glucose was used only for islet isolation. Krebs Ringer Buffer (KRB) solution was prepared with the following solutions, added in order: 100 ml of 0.9% NaCl; 4 ml of 11.5 mg/ml KCl; 1 ml of 21.1 mg/ml KH_2PO_4; 1 ml of 38.2 mg/ml $MgSO_4$; 3 ml of 16.1 mg/ml $CaCl_2$; and 15 ml of 13 mg/ml $NaHCO_3$. After stirring, excess fluid was poured off to leave a final volume of 100 ml. Three grams of powdered albumin (Fraction V. #17C0085, Sigma) was added to the solution. $NaHCO_3$ (13 mg/ml) was used to bring the pH to 7.4.

All prostaglandin solutions were made up as 100% ethanol

stock solutions of PGE_2 (10^{-3}, 10^{-4}, and 10^{-5} M) and Interoxaphenylene-PGE_1, or U51095 (10^{-4}, 10^{-5}, and 10^{-6} M). These solutions were stored at -70°C until needed. Ten minutes before the beginning of the experimental period, five microliters of the specific prostaglandin solution were added to the particular experimental vial already containing one milliliter of KRB. The final concentrations of the solutions were 10^{-5}, 10^{-6}, and 10^{-7} M of PGE_2 and 10^{-6}, 10^{-7}, and 10^{-8} M of U51095.

A stock solution of 1000 uM aspirin was prepared prior to the experiments. Ten minutes before the beginning of the pre-incubation and incubation periods of the aspirin experiments, 0.1 ml of 1000 uM aspirin was added to 0.9 ml KRB to yield a final concentration of 100 uM aspirin.

Tests and Test Solutions

The specific tests for insulin release by islets and the sequence of solutions used are described below:

In order to test the effect of PGE_2 on islets, the prostaglandin was administered under two experimental conditions - a non-stimulatory level of glucose and a stimulatory glucose level:

PGE_2 with Low Glucose Incubation:

Pre-Incubation Medium: KRB + 30 mg% glucose

Experimental Medium: KRB + 30 mg% glucose + 10^{-5} M, 10^{-6} M, or 10^{-7} M PGE_2

Control Medium: KRB + 300 mg% glucose

KRB + 30 mg% glucose

PGE_2 with High Glucose Incubation:

Pre-Incubation Medium: KRB + 30 mg% glucose

Experimental Medium: KRB + 300 mg% glucose and 10^{-5} M, 10^{-6} M, or 10^{-7} M PGE_2

Control Medium: KRB + 300 mg% glucose

KRB + 30 mg% glucose

In order that the effects of PGE_2 could be compared to those of U51095 (Interoxaphenylene-PGE_1), the latter compound was administered under similar conditions:

U51095 with Low Glucose Incubation:

Pre-Incubation Medium: KRB + 30 mg% glucose

Experimental Medium: KRB + 30 mg% glucose + 10^{-6} M, 10^{-7} M, or 10^{-8} M U51095

Control Medium: KRB + 300 mg% glucose

KRB + 30 mg% glucose

U51095 with High Glucose Incubation:

Pre-Incubation Medium: KRB + 30 mg% glucose

Experimental Medium: KRB + 300 mg% glucose + 10^{-6} M, 10^{-7} M, or 10^{-8} M U51095

Control Medium: KRB + 300 mg% glucose

KRB + 30 mg% glucose

An inhibitor of prostaglandin synthesis, aspirin, was tested to examine whether it would affect the insulin release:

Aspirin with Low or High Glucose Incubation:

Pre-Incubation Medium: KRB + 30 mg% glucose

Rinse Medium: KRB + 30 mg% glucose

Experimental Medium: KRB + 150 mg% glucose + 100 uM aspirin

KRB + 300 mg% glucose + 100 uM aspirin

Control Medium: KRB + 30 mg% glucose in both pre-incubation and experimental periods

KRB + 30 mg% glucose in pre-incubation period; KRB + 300 mg% glucose in experimental period

KRB + 30 mg% glucose + 100 uM aspirin in pre-incubation period; KRB + 300 mg% glucose in experimental period

Preparation of Culture Hardware

Test tubes (13 x 100 mm Dispo-Culture, Kimble) were soaked for 10 minutes in 1% silicone-water solution (Siliclad, Clay-Adams)

and air dried. One dram glass vials (Opticlear, Kimble) were boiled in distilled water for 30 minutes, rinsed in alcohol, rinsed with acetone, and air dried.

Glass loop holders were prepared from Pasteur Pipettes (5 3/4", Dispo-Capillary, Scientific Products) broken off two inches from the tip. The smaller end was filled with paraffin wax. The loops were formed from glass rods (1 mm diameter, Corning) by heating and stretching until a needle-thin (25 gauge) diameter was achieved. These thin rods were held with hemostats and softened in a flame while using a needle to push the end into a loop. The loop was immediately removed from the flame and the rod was broken off two inches from the looped end. The loops were attached to their holders by warming the paraffin-filled ends of the pipettes and pushing the straight end of the loop into the pipette before cooling.

Islet baskets consisted of a plastic tube covered with nylon mesh on one open end. The mesh was held in place by a plastic sleeve. Nylon mesh used in the preparation of the islet baskets was first boiled in 0.5% $NaHCO_3$ solution for thirty minutes, followed by boiling in distilled water for thirty minutes. It was then air dried.[17]

The body of the basket was prepared using two sizes of plastic pipetter tips: 10-200 ul (#9025, Dispo MLA) and 250-1000 ul (#9026, Dispo MLA). The plastic sleeve was created by sawing a five millimeter piece from the large end of the 250-100 ul pipette tip.

The main part of the basket body was made by sawing the smaller pipette tip approximately in half to leave a tube 15 mm long. Each of these pieces was trimmed with a scalpel to remove extraneous bits of plastic.

The nylon mesh was cut into 10 mm squares and placed on top of the plastic ring. The plastic tube was forced into the ring, pushing the mesh down to form a cover. The excess mesh on the side of the basket was trimmed away.

PROCEDURE

Each experiment was performed using the islets from one animal, male or female (Upjohn strain) with weights ranging from 120-170 grams. After killing the rat, an incision was made through the skin and abdominal muscle extending from the urogenital region anterior to the thoracic cavity. The pancreas was located on the mesentery along the lower third of the large intestine and clipped away from it until reaching the small intestine and the pylorus. A second line of separation began at the spleen and ended at the stomach. The pancreas was finally separated at the pylorus and placed in approximately five milliliters of HBSS containing 50 mg% glucose. The less dense fatty tissue was easily located and removed.

The pancreas tissue was minced with dissecting scissors in 5 ml of HBSS containing 50 mg% glucose and poured into a siliconized test tube. The supernatant was aspirated and the minced tissue rinsed again. This process was repeated 2-3 times until the supernatant appeared clear. The final solution consisted of approximately one milliliter HBSS with 50 mg% glucose and minced pancreas. To this was added 20-25 mg of collagenase (Type IV, Boehringer, Lot #1027419 and later Type IV, Worthington, Lot #CLS47B177P). The test tube was stoppered and held in a 37° C water bath with vigorous handheld shaking for a least five minutes until the solution had a milky appearance devoid of pieces of tissue.

Approximately 6 ml of HBSS with 50 mg% glucose was added to the dispersed tissue in solution and the mixture was centrifuged at 500 rpm for 10 seconds (International Centrifuge, model CL, International Equipment Company). The supernatant was poured off and the pellet resuspended in 6 ml HBSS containing 50 mg% glucose. The centrifugation was repeated. The entire process was repeated 2-3 times until the supernatant appeared clear.

Six milliliters of HBSS containing 50 mg% glucose and 3% bovine serum albumin (sterile solution, Sigma, Lot #16C0238) was added to the final pellet of pancreatic tissue. The mixture was centrifuged as above and the supernatant fraction removed. The tissue was rinsed again with the albumin solution, centrifuged, and the supernatant removed. Finally, the pellet was resuspended in 1.5 ml of this solution.

Islet isolation wells were prepared by placing one ml of HBSS containing 50 mg% glucose and 3% albumin in each well of Spot Reaction Plates (Black, #1, Coors). Approximately 0.1 ml of the islet solution was added to nine of the twelve prepared wells. The other wells were used for collection of the islets. The plate was placed under a dissection scope at 16 x magnification. A 100 ul micropippetor (Micro Clinac, LaPine, and Kimax) was used for plucking islets away from the surrounding tissue. The islets are distinguished from the grayish-white scattered pieces of acinar tissue as ovoid, white shapes. Using the

micropippetor, the islets were pulled up into the pipette and dispensed into one of the empty wells of each plate. This procedure was repeated until as many islets as possible were gathered into one or two collection wells per plate.

The extra solution in the wells containing the collected islets was removed using absorbent paper. The other wells were cleaned and 1 ml of fresh HBSS with 50 mg% glucose and 3% albumin was added to each well. The prepared glass loops were used to pluck the islets individually from the previously collected group. Each islet was transferred to another clean well containing the same medium until groups of seven islets were completed.

Each group of seven islets was placed in a separate islet basket using the 100 ul pippetor. The baskets were then placed in the one dram vials which contained the appropriate incubation medium. During both the pre-incubation period of 45 minutes and the experimental period of either 4 or 30 minutes, the set of vials was enclosed in a 37° C water bath shaker (American Instrument Company, #5-3974) and the surrounding air was gassed with 95% air-5% CO_2.

At the end of the pre-incubation period, the individual baskets were removed from the vials and the liquid drained by gravity flow back into the vials. Each basket was then placed in the experimental vial for the prescribed time. In the aspirin experiments, the baskets were first dropped into a second pre-

incubation medium containing KRB and 30 mg% glucose before being placed in the experimental medium. In some of the aspirin experiments, this step was not done in order to determine whether rinsing has an effect on insulin release. After the experimental period was over, the baskets were removed, drained, and discarded. The medium in the vial was frozen and stored at -70° C until insulin could be measured.

Radioimmunoassay for Insulin

The radioimmunoassay for insulin was performed according to the method described by Zaharko and Beck[18] and developed by B. Wyse (Upjohn Laboratories, Kalamazoo, MI). The buffer was prepared using the following reagents: 13.22 grams Trizma-HCl (Sigma); 0.97 grams Trizma-Base (Sigma); and 30.33 ml Bovine Serum Albumin (30%, Armor). The volume of the buffer was adjusted using sterilized distilled-deionized H_2O to obtain a final volume of one liter at pH of 7.4. The buffer was used for all solutions, standards, and dilutions throughout the assay.

The insulin used for the standards was dissolved in dilute HCl (pH 2.5-3.0) at a concentration of 1 mg/ml (Dr. M. Root, Lilly Research Labs, Indianapolis, IN 46206). Starting with a 512 uU/tube concentration, the standards were prepared by serially diluting (1:2) this tube until a tube with a concentration of 1 uU/tube was reached. The standards were organized into sets

(0.5 ml/tube) and frozen at -20° C until needed.

The antibody to insulin had been prepared previously to this study by immunizing guinea pigs with bovine insulin. The final dilution of the serum (Upjohn anti-insulin antibody diluted 1:80,000) bound 60-70% of the 4-5 uU insulin after two hours incubation at 25°C.

I^{125} insulin was diluted with buffer after it arrived to a concentration of 100 uU/ml and frozen in 5 ml aliquots (Nuclear International Corporation, 204 Second, Waltham, MA 02514). The insulin was thawed as needed and diluted to 10 uU/tube for use in the assay.

The cellulose slurry used to separate the unbound fraction of insulin required a 1:10 weight/volume dilution of cellulose powder (Brinkman, MN300) with buffer. The slurry was prepared at least one hour before it was needed and stirred constantly.

The total incubation volume for this assay was 1.5 ml. All standards and samples were contained in a volume of 0.5 ml and performed in duplicate. Also included in the assay were tubes containing only buffer and I^{125} insulin to serve as total counts tubes. Other tubes with I^{125} insulin, buffer, and slurry gave the non-specific binding results. Finally, test tubes for the zero dose contained I^{125} insulin plus antibody plus slurry.

To each sample (0.5 ml) tube was added 0.5 ml of appropriately diluted insulin antibody. The same volume (0.5 ml) of I^{125} containing 4-5 uU/tube was then added. The contents

of the tubes were mixed and incubated at 25° C for two hours. At the end of the incubation period, one ml of cellulose slurry was added to each tube and the contents centrifuged for four minutes. The supernatant was separated from the precipitate and only the precipitate, containing the unbound insulin, was counted in a gamma counter for one minute (counting at least 10,000 counts). The resulting values were used in the RODBARD - RIA computer program to determine the Standard Curve for each assay and the amount of insulin released by the islets during the various incubation periods.

Statistics on the results were performed using the Biostatistics Department Stat Pac. The Student's t-test was performed for the test of significance between the pre-incubation period and the experimental period insulin release. One Way Analysis of Variance was performed for the test of significance between the control groups and the treatment groups of insulin release. In both tests, p values of less than .05 indicated significant differences between the groups tested. Bartlett's chi-square test was used to measure the homogeneity of variance and the Kruskal-Wallis test checked for the equality of the means.

RESULTS

Minimal insulin release was observed in the presence of 30 mg% glucose and 150 mg% glucose, but stimulation of insulin release was seen in the higher levels of glucose administration (Figure 1, Table I). Maximal levels of insulin response occurred at 600 mg% glucose, since there was no significant difference between the response at 600 mg% glucose and 1000 mg% glucose.

Prostaglandin E_2 (PGE_2) had no effect on basal insulin release in the presence of 30 mg% glucose (Figure 2). There was also no significant difference between the insulin responses with various concentrations of PGE_2 in the experimental medium and the low glucose control levels of insulin (Table II). However, when islets were exposed to PGE_2 and a stimulatory level of glucose (300 mg%), a significant decrease was observed in insulin release from treated islets compared to control islets (Table II). There was no real dose response to PGE_2 at the various concentrations (10^{-5} M, 10^{-6} M, or 10^{-7} M); the inhibition of insulin response was maximal at 10^{-6} M PGE_2 but not significantly different from the others (Figure 3).

A study undertaken to determine if PGE_2 exerts its effect on the primary peak of biphasic insulin release revealed that there was no inhibition of insulin release within the first few minutes (Figure 4). The amount of insulin release in the first four

minutes has been converted in the table and graph to microunits of insulin released per ten minutes for comparison with the other results (Table III). There was no dose response among the three concentrations of PGE_2 and the amount of insulin released.

U51095 did not affect the levels of insulin released in the presence of 30 mg% glucose (Figure 5). The basal levels of insulin remained essentially unchanged in the experimental medium containing any of the three U51095 concentrations (10^{-6} M, 10^{-7} M, or 10^{-8} M) in comparison with the low glucose control levels (Table IV). In a similar manner as PGE_2, the U51095 compound did inhibit glucose-stimulated (300 mg%) insulin release from islets (Figure 6). Again, there was no dose response observed among the various U51095 concentrations (Table IV). Finally, there was no significant difference between the response of PGE_2-treated islets and U51095-treated islets at any dose of either compound.

When administered in both the pre-incubation and experimental periods, aspirin (100 uM) exerted a significant effect on insulin release in the presence of 300 mg% glucose (Figure 7). It had no effect on insulin release with 150 mg% glucose over basal levels of low glucose control response. The amount of insulin released when islets were exposed to aspirin in the pre-incubation period but rinsed before being stimulated with glucose (300 mg%) was not significantly different from high glucose control levels with no aspirin exposure (Table V). All islets in this set of experiments

were rinsed before being placed in the experimental medium.

Islets exposed to aspirin in the pre-incubation period but not rinsed before the experimental period, during which they were exposed to 300 mg% glucose alone, showed a significant inhibition of insulin release (Table V). Aspirin also inhibited the response of islets to 300 mg% glucose with medium containing aspirin (100 uM) in both time periods, without rinsing the islets in-between. However, aspirin had no effect on the amount of insulin released at 150 mg% glucose compared to the low glucose control levels (Figure 8).

EXPLANATION OF TABLES AND GRAPHS

Each bar graph represents the actual microunits of insulin released for ten minutes per seven islets. The experimental medium insulin response levels are compared with their own pre-incubation medium levels of insulin. Each n value represents the number of groups of seven islets each.

The tables show only the difference between the experimental insulin response and the basal insulin response from the pre-incubation period. This method accounts for differences among individual islets and experimental preparations.

Every experiment had its own low and high glucose controls. Most of the experimental results are compared to the insulin response of high glucose control levels (300 mg%). Low glucose control values (30 mg%) are given to assure that there was a threshold level of glucose required for stimulation of insulin release in each experiment.

Figure 1: Effect of Various Glucose Concentrations on Insulin Release.

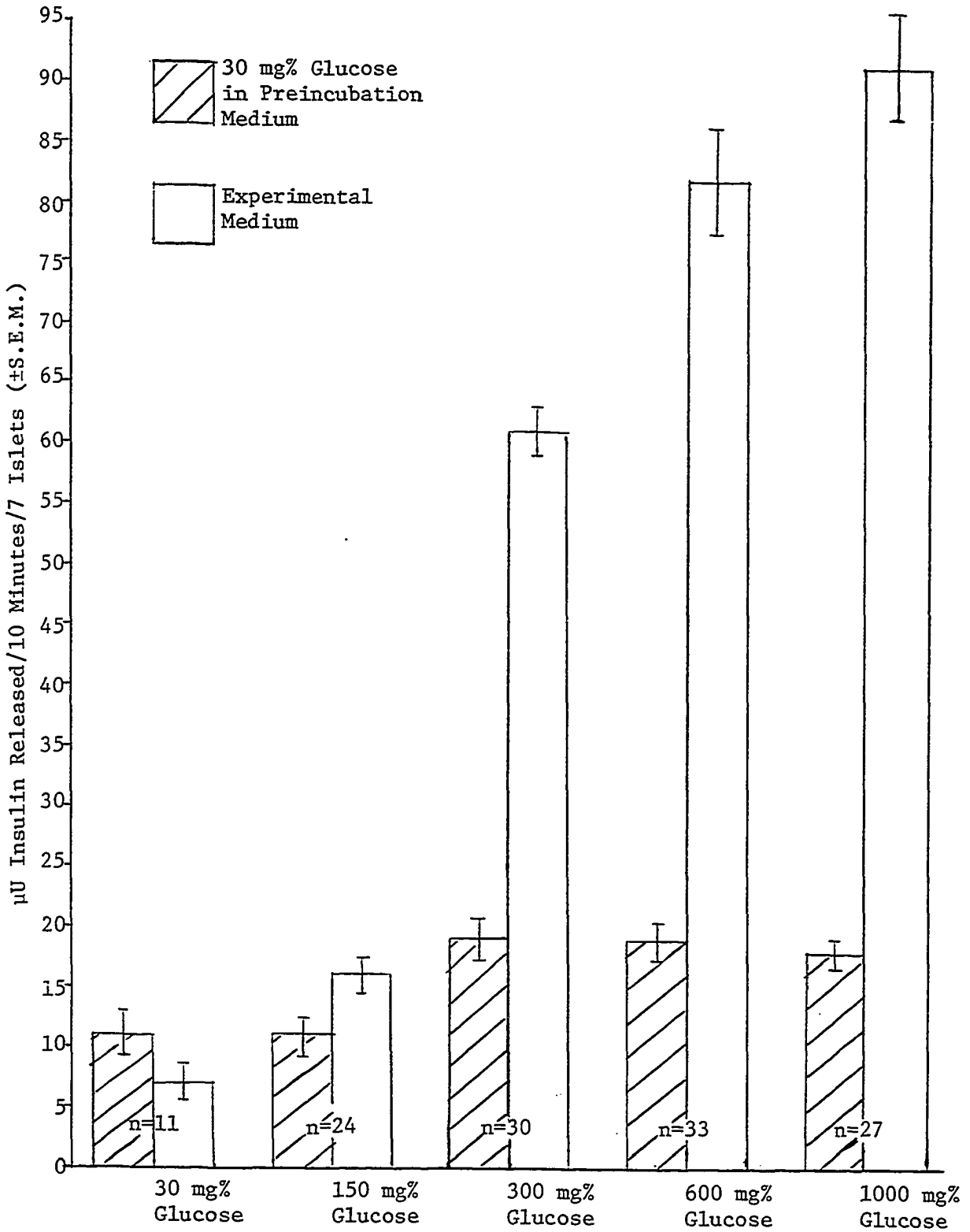

TABLE I. Effect of Various Concentrations of Glucose[1] on Insulin Release in the Presence of Glucose for Thirty Minutes

n_A^2	A [Glucose] Experimental Medium (mg%)	n_B^2	B [Glucose] Experimental Medium (mg%)	A^3 Insulin Response (μU/10 min)	B^3 Insulin Response (μU/10 min)	P^4
24	150	11	30	-5.22 ± 1.23	-4.74 ± 2.50	.080
30	300	24	150	41.20 ± 3.70	-5.22 ± 1.23	.000
33	600	30	300	64.30 ± 4.41	41.20 ± 3.70	.000
27	1000	33	600	73.50 ± 4.56	64.30 ± 4.41	.090

[1]30 mg%, 150 mg%, 300 mg%, 600 mg% or 1000 mg% glucose

[2]The number of groups of seven islets each.

[3]Each value represents the mean of: (insulin response in experimental medium) - (insulin response in preincubation medium). (± S.E.M.)

[4]Comparison of A with B.

Figure 4: Effect of PGE_2 on Early Release of Insulin by Glucose Stimulation.

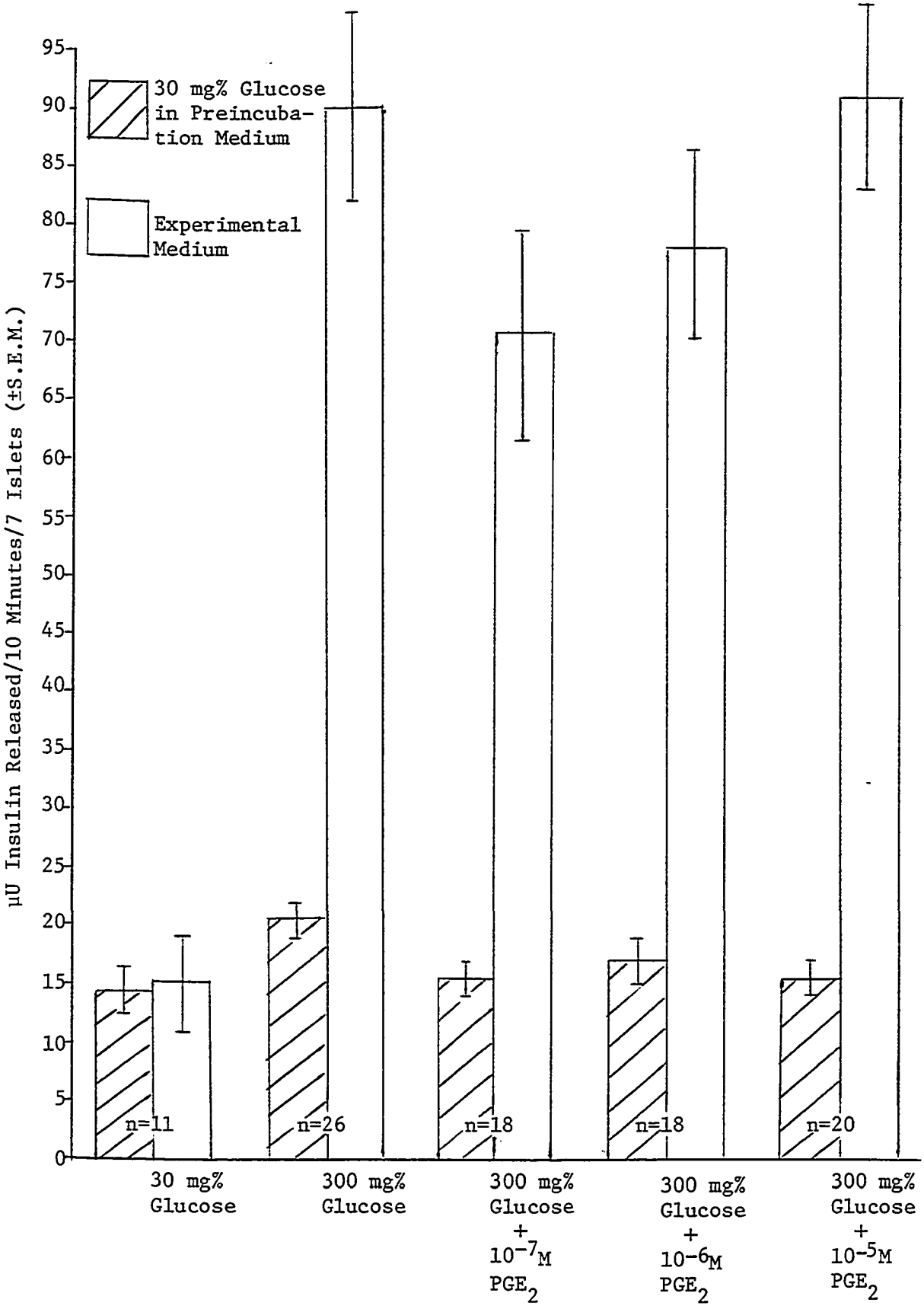

TABLE II. Effect of Prostaglandin E_2 (PGE_2) on Insulin Release in the Presence of Glucose (30 mg% or 300 mg%) for 30 Minutes

n[1]	[Glucose] (mg%) Experimental Medium	Experimental Medium	High Glucose[2] Control Insulin Response (μU/10 min)	Experimental[2] Insulin Response (μU/10 min)	P[3]
			(n=24)		
20	300	10^{-7} M PGE_2	66.20 ± 5.23	40.00 ± 6.34	.000
28	300	10^{-6} M PGE_2	66.20 ± 5.23	25.50 ± 2.64	.000
20	300	10^{-5} M PGE_2	66.20 ± 5.23	31.20 ± 3.92	.000
6	30	--	66.20 ± 5.23	.65 ± 1.83	.000
			(n=10)		
22	30	10^{-7} M PGE_2	43.40 ± 5.61	-0.11 ± 1.47	.000
22	30	10^{-6} M PGE_2	43.40 ± 5.61	-2.00 ± 0.82	.000
23	30	10^{-5} M PGE_2	43.40 ± 5.61	-0.77 ± 1.50	.000
15	30	--	43.40 ± 5.61	-2.20 ± 1.40	.000

[1]The number of groups of seven islets each.

[2]Each value represents the mean of: (insulin response in experimental or control medium) - (insulin response in preincubation medium). (± S.E.M.)

[3]Comparison of high glucose insulin response (300 mg%) with experimental medium response.

Figure 2: Effect of PGE_2 on Low Glucose Stimulation of Insulin Release.

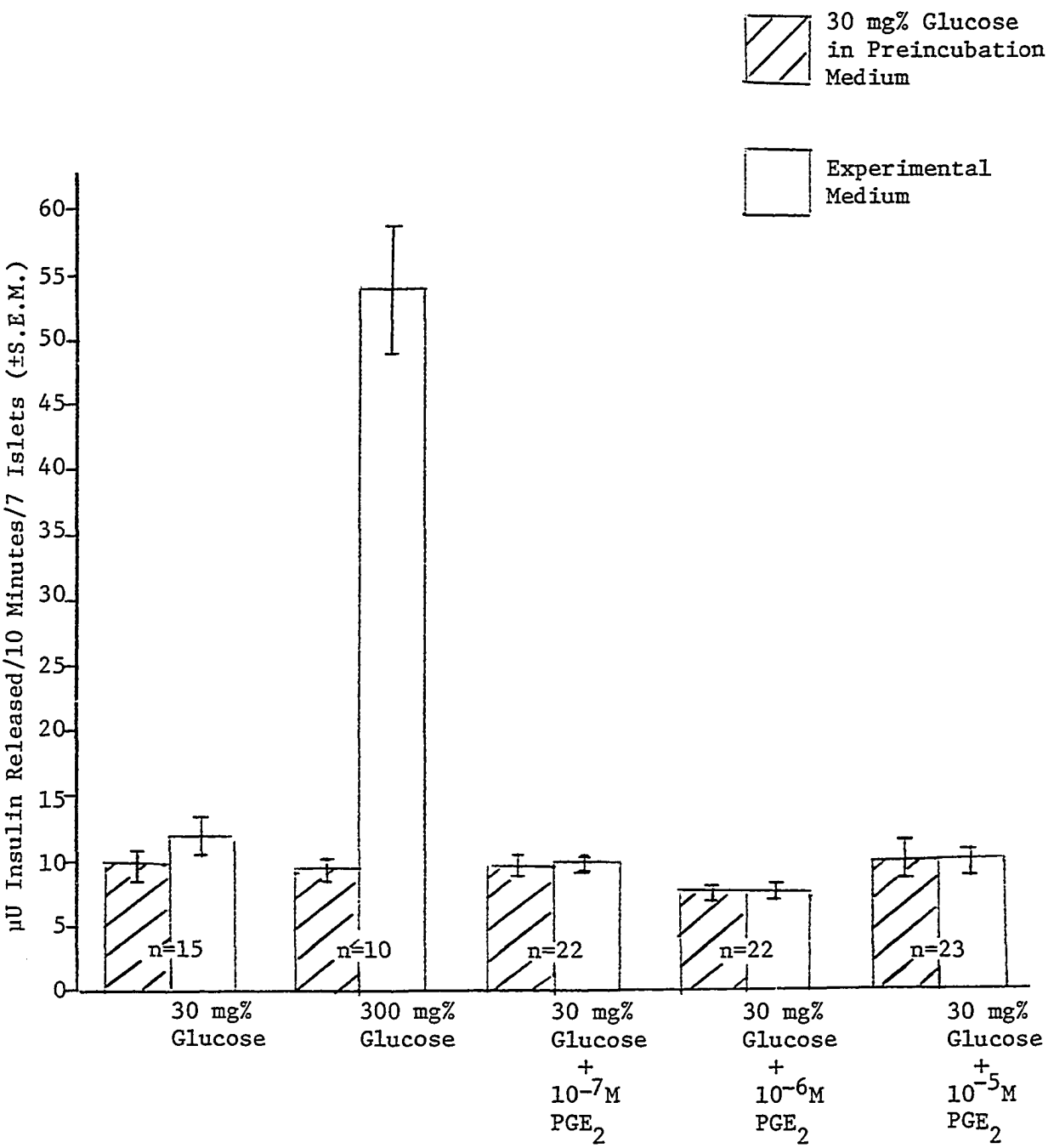

Figure 3: Effect of PGE_2 on High Glucose Stimulated Insulin Release.

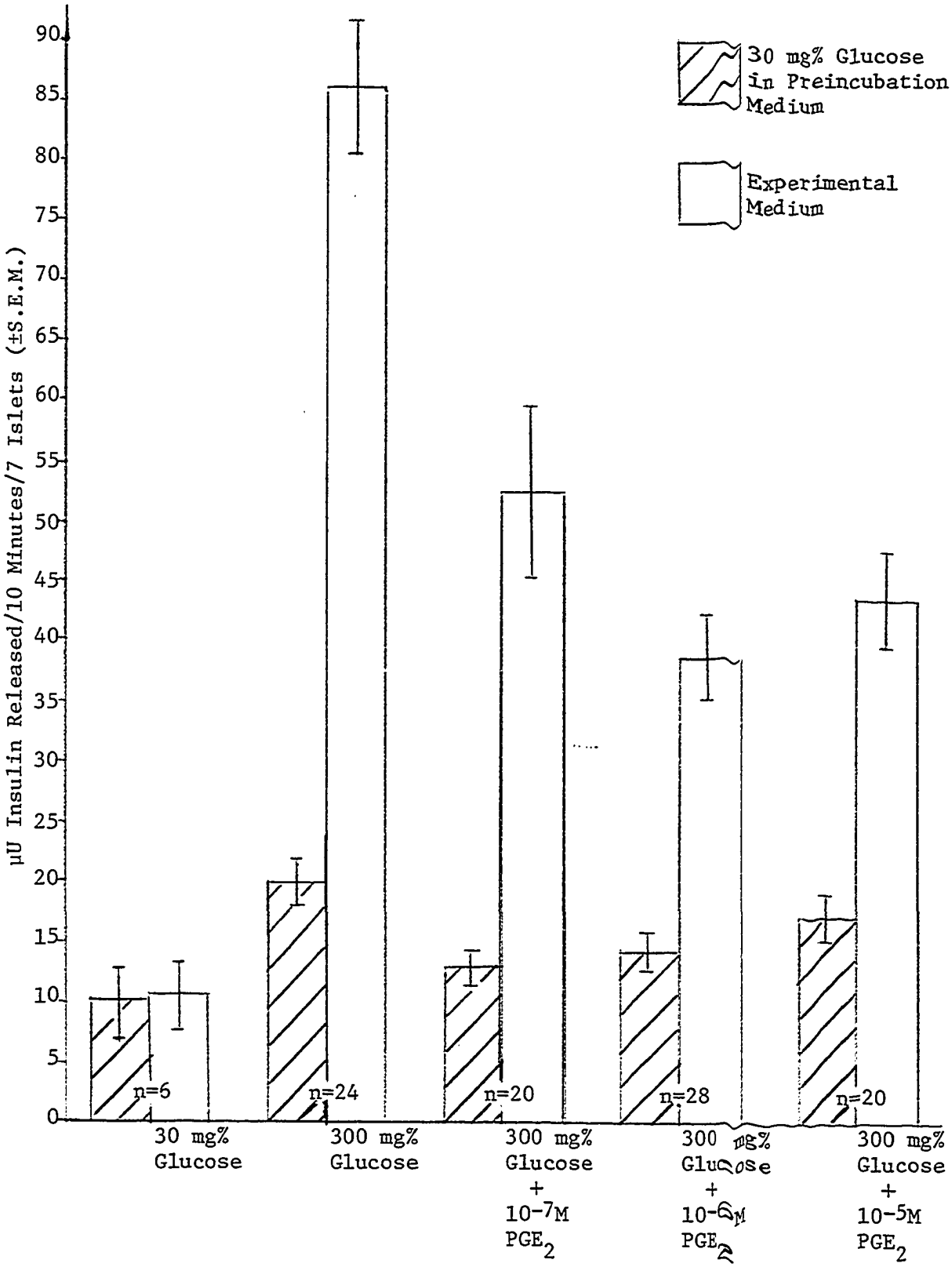

TABLE III. Effect of Prostaglandin E_2 (PGE_2) on Insulin Release in the Presence of Glucose (300 mg%) for Four Minutes

n[1]	Experimental Medium	High Glucose[2] Control Insulin Response (μU/10 min) (n=26)	Experimental[2] Insulin Response (μU/10 min)	P[3]
18	10^{-7} M PGE_2	69.70 ± 7.15	55.50 ± 8.91	.687
18	10^{-6} M PGE_2	69.70 ± 7.15	60.60 ± 7.07	.387
20	10^{-5} M PGE_2	69.70 ± 7.15	73.80 ± 7.09	.687
11	30 mg% glucose	69.70 ± 7.15	14.97 ± 1.87	.000

[1]The number of groups of seven islets each.

[2]Each value represents the mean of: (insulin response in experimental medium or control medium) - (insulin response in preincubation medium). (± S.E.M.)

[3]Comparison of high glucose control insulin response (300 mg%) with experimental medium response.

Figure 5: Effect of U51095 (IOP-PGE$_1$) on Low Glucose Stimulated Insulin Release.

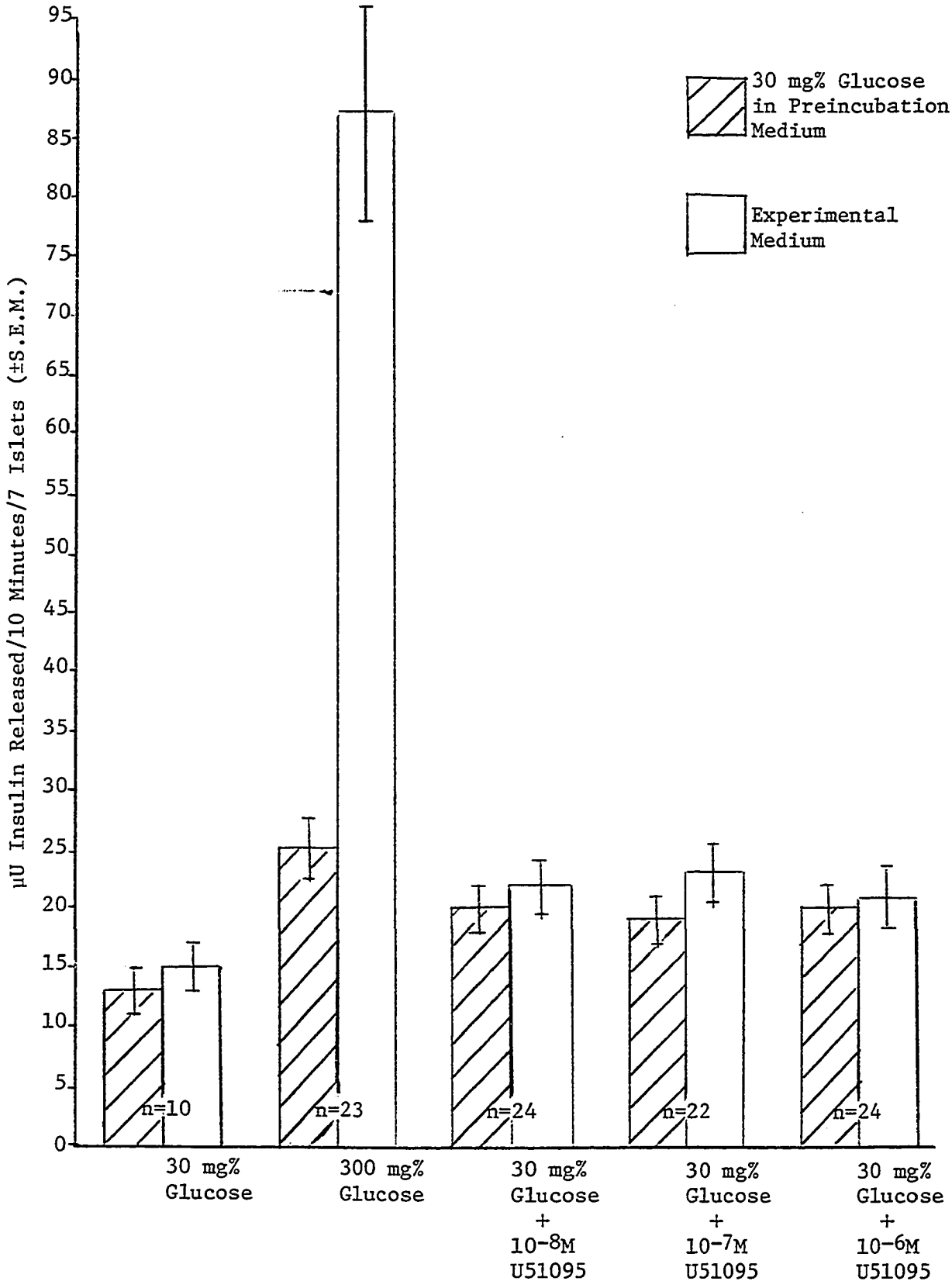

Figure 6: Effect of U51095 (IOP-PGE_1) on High Glucose Stimulated Insulin Release.

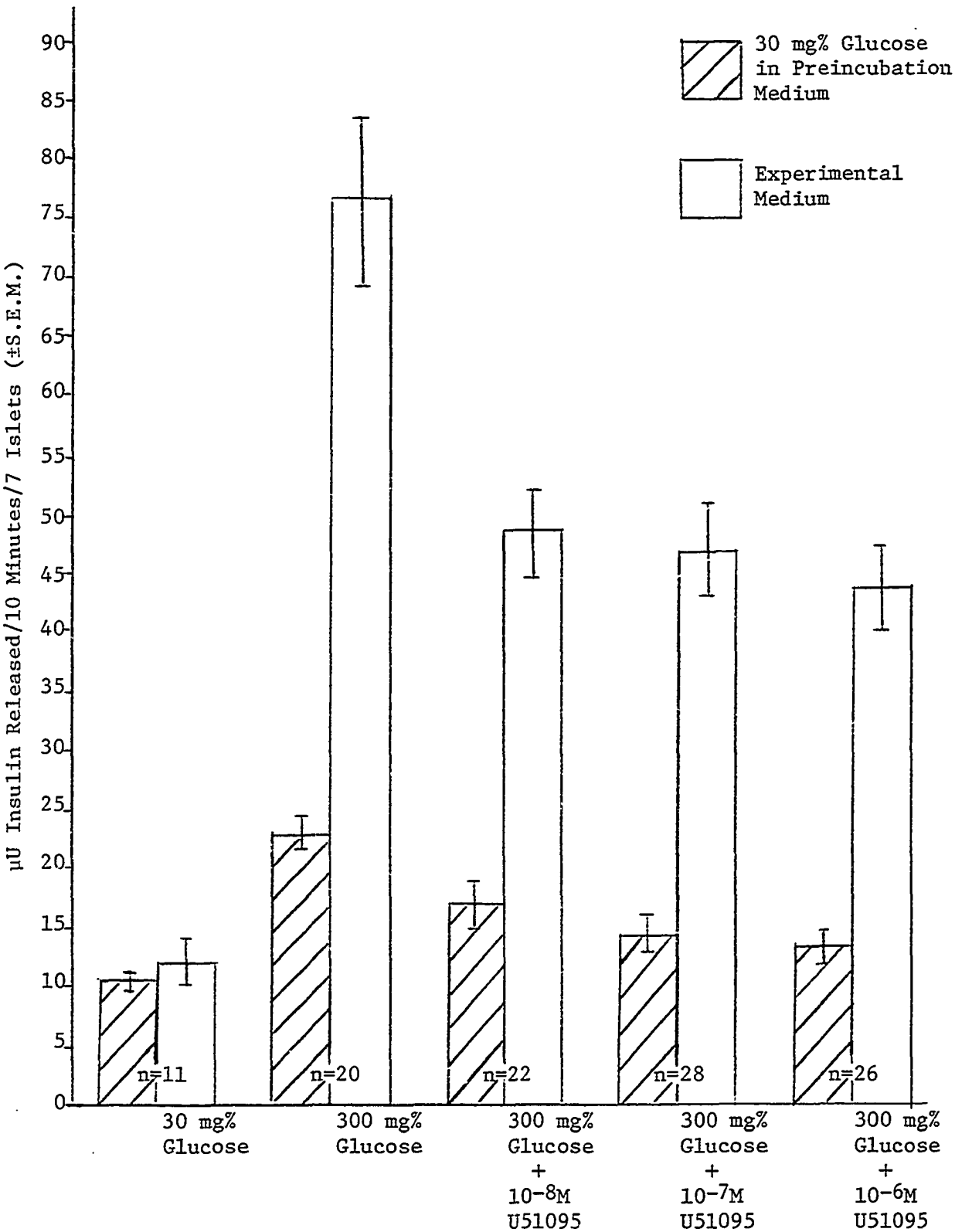

TABLE IV. Effect of Interoxaphenylene-PGE_1 (U-51,095) on Insulin in the Presence of Glucose (30 mg% or 300 mg%) for Thirty Minutes

n[1]	[Glucose] (mg%) Experimental Medium	Experimental Medium	High Glucose[2] Control Insulin Response (μU/10 min)	Experimental[2] Insulin Response (μU/10 min)	P[3]
			(n=20)		
22	300	10^{-8} M U-51,095	53.10 ± 6.60	30.45 ± 3.49	.007
28	300	10^{-7} M U-51,095	53.10 ± 6.60	32.32 ± 3.73	.002
26	300	10^{-6} M U-51,095	53.10 ± 6.60	30.23 ± 2.89	.024
11	30	--	53.10 ± 6.60	2.05 ± 1.76	.000
			(n=23)		
24	30	10^{-8} M U-51,095	61.60 ± 8.37	1.91 ± 1.62	.000
22	30	10^{-7} M U-51,095	61.60 ± 8.37	3.15 ± 1.06	.000
24	30	10^{-6} M U-51,095	61.60 ± 8.37	0.53 ± 1.24	.000
10	30	--	61.60 ± 8.37	1.98 ± 3.30	.000

[1]The number of groups of seven islets each.

[2]Each value represents the mean of: (insulin response in experimental or control medium) - (insulin response in preincubation medium). (± S.E.M.)

[3]Comparison of high glucose control insulin response (300 mg%) with experimental medium response.

Figure 7: Effect of Aspirin on Glucose Stimulated Insulin Release Islets Rinsed in KRB Containing 30 mg% Glucose Only After Exposure to Aspirin in Preincubation Period.

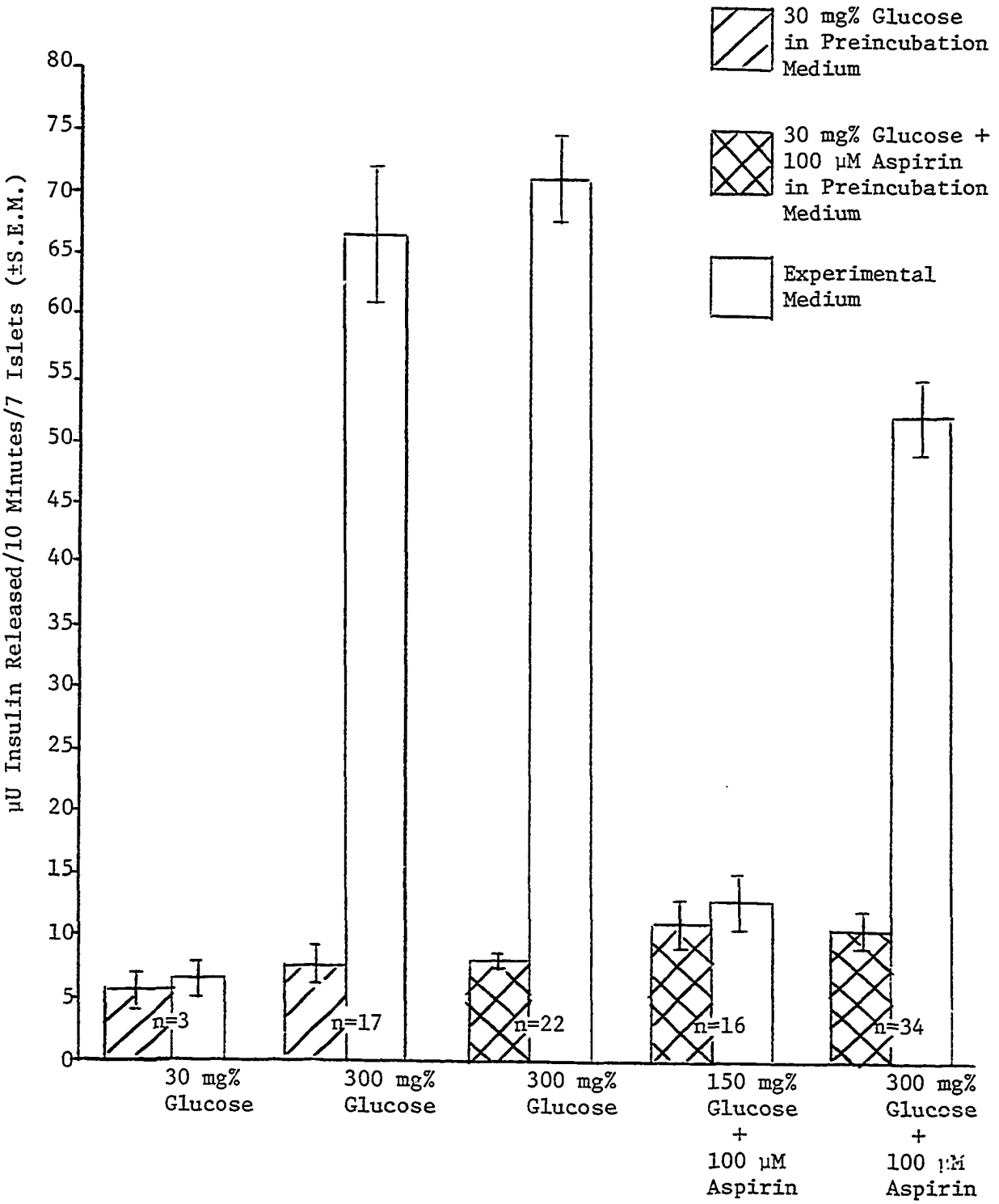

Figure 8: Effect of Aspirin on Glucose-Stimulated Insulin Release Without Rinsing.

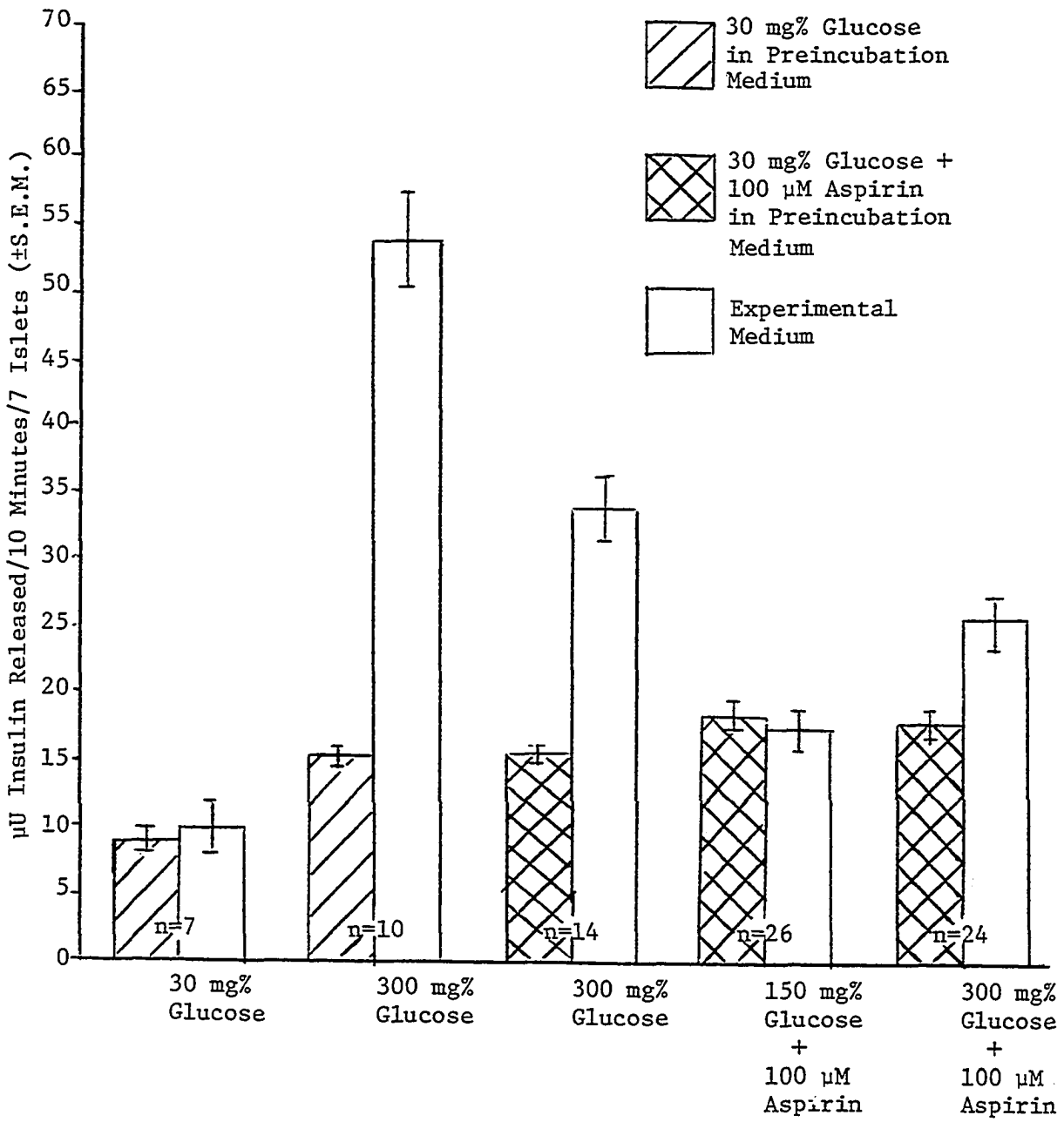

TABLE V. Effect of 100 μM Aspirin on Insulin Release in the Presence of Glucose (150 mg% or 300 mg%) for Thirty Minutes With[1] and Without[2] Rinsing

n[3]	Preincubation Medium (in addition to 30 mg% glucose)	[Glucose](mg%) Experimental Medium	Experimental Medium	High Glucose[4] Control Insulin Response (μU/10 min)	Experimental[4] Insulin Response (μU/10 min)	P[5]
RINSED ISLETS				(n=17)		
25	100 μM Aspirin	300	--	58.40 ± 5.81	63.20 ± 4.01	.394
16	100 μM Aspirin	150	100 μM Aspirin	58.40 ± 5.81	2.26 ± 1.89	.000
34	100 μM Aspirin	300	100 μM Aspirin	58.40 ± 5.81	40.40 ± 2.67	.001
3	--	30	--	58.40 ± 5.81	0.37 ± 0.19	.000
UNRINSED ISLETS				(n=10)		
14	100 μM Aspirin	300	--	37.90 ± 3.78	18.40 ± 2.47	.000
26	100 μM Aspirin	150	100 μM Aspirin	37.90 ± 3.78	-3.06 ± 0.95	.000
24	100 μM Aspirin	300	100 μM Aspirin	37.90 ± 3.78	18.20 ± 3.23	.000
7	--	30	--	37.90 ± 3.78	-1.23 ± 2.33	.000

[1]The islets were rinsed in Krebs-Ringer-Buffer containing 30 mg% glucose after exposure to aspirin in preincubation period and before the experimental period.
[2]The islets were not rinsed before the experimental period.
[3]The number of groups of seven islets each.
[4]Each value represents the mean of: (insulin response in experimental or control medium) - (insulin response in preincubation medium). (± S.E.M.)
[5]Comparison of high glucose control insulin response (300 mg%) with experimental medium insulin response.

DISCUSSION

Each experimental set measured low and high glucose control levels of insulin release. While all the islets exposed to 30 mg% glucose in both time periods responded similarly, there was considerable variance among the control values for the glucose-stimulated insulin response using Krebs-Ringer Buffer containing only 300 mg% glucose. There are several reasons why these values may have differed. The collagenase used for digestion of the pancreatic tissue varied in enzymatic efficiency. The time required for good digestion ranged from five minutes to over ten minutes. This prolonged exposure could have affected the cell membranes of the islet structure itself to produce the variations in response. Also, there could be differences among individual rats depending on their age and diet which may indirectly affect the response of islets to glucose stimulation. Finally, slight variation in experimental technique may have contributed to the overall differences in values. In order to account for this variation, the difference between insulin released during the pre-incubation period and experimental period was measured for each group of islets. Then, within each set of experiments, the difference between these time periods of the high glucose control islets was compared to the change in insulin release during the same time periods of the various treatment groups. In this way, the relative variation from one group to the next was minimized.

In an effort to clarify the role of PGE_2 in insulin secretion, isolated rat islets were exposed to the prostaglandin for a set period of time in the presence or absence of glucose stimulation. The insulin released was then measured by radioimmunoassay. In addition, the effect of aspirin, a prostaglandin synthetase inhibitor, on insulin secretion was also examined in a similar manner. Correlation of this data with other experiments should help determine whether prostaglandins and aspirin influence insulin release.

Varying the glucose concentrations in media containing isolated islets resulted in significant increases in insulin release in response to increasing levels of glucose. While no extra insulin was released above basal levels when islets were incubated with 30 mg% or 150 mg% glucose in Krebs-Ringer-Buffer (KRB), there was strong stimulation of insulin secretion at the higher doses of 300 mg%, 600 mg%, and 1000 mg% glucose. Since 1000 mg% glucose is 0.056 M, this is probably a pharmacological dose causing hyperosmotic damage to the islets. But the results using 300 mg% and 600 mg% glucose in KRB indicate that 300 mg% glucose is not the maximal level of stimulation found in some *in vitro* experimental work such as tests with Chinese hamster islets (Kupiecki, F., personal communication). Therefore, if either of the prostaglandins or the aspirin had enhanced insulin release, it would have been detectable since a maximum stimulatory concentration of glucose had not been reached using 300 mg% glucose in the high glucose experimental medium. Since there was no significant difference between 600 mg% and 1000 mg% glucose stimulation of insulin release, it is assumed that the maximum level

of glucose stimulation occurs at 600 mg% glucose in the medium.

Since it was established that a submaximal yet stimulatory level of glucose to produce significant insulin release was KRB-containing 300 mg% glucose, the effect of the prostaglandins on inhibition of potentiation of insulin release could be tested. The effect that PGE_2 had on rat islet insulin release during a thirty minute exposure was in agreement with Sacca[3], Robertson[4], and Burr[5] who also showed decreased insulin response when PGE_2 was added to the incubation medium containing high glucose (300 mg%). Konturek[19] and co-workers had similar results in the tests they performed on human volunteers. While there was no change in basal insulin release after administration of PGE_2, there was a decreased response to glucose stimulation. Their group believed that the prostaglandin exerts a direct effect on the islet beta cells and that it is not merely an interference with absorption of glucose in the gut. However, since Konturek's study was performed in vivo, several endogenous factors may enter into their results, such as release of other inhibitors, so that the effect of PGE_2 may have been indirect. There was no dose response to PGE_2 between 10^{-5} M and 10^{-7} M PGE_2 in the present study, indicating that even 10^{-7} M PGE_2 provided maximum inhibition of islet insulin secretion.

In order for PGE_2 to exert any effect on the islets, there must be a concomitant glucose-stimulated insulin release. This was shown by a lack of effect on the levels of insulin released from islets exposed to PGE_2 in the absence of a stimulatory glucose concentration. Most previous studies also demonstrate a similar re-

sult with non-stimulatory glucose levels[5,6,15]. It is possible to conclude from this finding that the action of PGE_2 is to alter the response of the islets to glucose stimulation but not to completely block the release of insulin.

After observing the inhibitory effect of PGE_2 on islets treated for thirty minutes with the prostaglandin and 300 mg% glucose in KRB, another set of experiments was performed in which the islets were exposed to PGE_2 for four minutes instead of thirty minutes. This was done to test whether PGE_2 affected the biphasic response of insulin release described by several investigators[7,8,9]. In all cases, the acute peak of insulin release occurs within the first four minutes following glucose stimulation. In this way, the effect of PGE_2 on the primary peak of insulin release would be observed. The results indicate a lack of effect of PGE_2 on the early release of insulin with glucose stimulation. There was no dose response from 10^{-5} M to 10^{-7} M PGE_2, so that either 10^{-5} M was too low to yield an effect within that time period, or the action of PGE_2 is exerted especially after the first four minutes of insulin release. The latter explanation seems more feasible since the effect of PGE_2 at dosages greater than 10^{-5} M may demonstrate a pharmacological response rather than a physiological response.

An analog of PGE_2, called U-51,095 (Interoxaphenylene-PGE_1) has recently been synthesized and is being tested alongside PGE_1 and PGE_2 for similarities in its mechanism of action, if any. Nishizawa[10] showed U-51,095 to be thirty times more potent than PGE_1 in studies on the inhibition of platelet aggregation. While PGE_1 re-

quired a dose of 0.03 μg/ml for effectiveness, U-51,095 inhibited the platelets at a concentration of 0.001 μg/ml. Since the effect of U-51,095 was in agreement with that of PGE_1 in the hematological study, it was expected that the synthetic prostaglandin would also agree with the effect of PGE_2 in the insulin experiments, though no previous work has been done in this area.

The results of the U-51,095 experiments did, in fact, support the results of the PGE_2 experiments. No effect on basal levels of insulin release was observed from islets exposed only to low glucose stimulation (30 mg%). However, at a concentration of 300 mg% glucose in KRB, U-51,095 significantly decreased the insulin response, similar to the effect of PGE_2 on the islets. Since U-51,095 mimics its PGE_2 analog, these results give added emphasis to the conclusion that PGE_2 decreases insulin release following glucose stimulation. Again there was no dose response observed between 10^{-6} M and 10^{-8} M U-51,095 so that the 10^{-8} M dose was already maximal. However, the potency of U-51,095 observed here was different from the platelet aggregation studies. In the present study, the potency of U-51,095 was similar to, but not greater than, the effect of PGE_2, inasmuch as there was no significant difference between groups of varying PGE_2 concentrations and varying concentrations of U-51,095.

The effect of prostaglandin and prostaglandin analog on insulin release may be expressed through changes in the cyclic AMP levels of the islet cells. The prostaglandins have been shown to be involved in the modification of cAMP levels of such tissues as the liver, salivary glands, and the stomach[20]. Several authors have

reported increases of cAMP concomitant with decreased platelet aggregation after exposure of the blood to PGE_2.[21,22,23] However, little work has been done on insulin release and cAMP. In one of the few studies measuring the effect of PGE_1 on cAMP levels of islet cells, Johnson *et al.*[1] demonstrated an increase in cAMP after PGE_1 administration along with an increase in insulin release using 300 mg% glucose stimulation. These results contradict the findings of the present study in which PGE_2 inhibits insulin response to glucose stimulation. It is difficult to compare the two experiments, however, since levels of cAMP were not measured in the present study and isolation techniques differed. Strong statistical evidence ($p < 0.001$) emphasizes the negative effect of PGE_2 and U-51,095 on islets of Langerhans.

Since the effects of both PGE_2 and U-51,095 are to decrease insulin response to glucose stimulation, it was of interest to observe the effect a blocker of prostaglandin synthesis had on insulin release. It was expected that the inhibition of prostaglandin synthesis would block the effect of endogenous PGE_2 so glucose-stimulated insulin response would not be inhibited. The effect of the blocker itself on insulin release could also be determined.

In view of the fact that aspirin is a prostaglandin synthetase inhibitor both *in vitro*[11] and *in vivo*[12], experiments using 100 μM aspirin were undertaken to determine whether the blockage of prostaglandin synthesis by this agent would affect insulin response. There was no effect on basal insulin release from islets exposed to 100 μM aspirin in the presence of low (150 mg%) glucose with either

rinsed or unrinsed islets. There was, however, a significant inhibition of insulin release from islets stimulated with 300 mg% glucose if they were exposed to 100 μM aspirin in either or both time periods. The effect of the aspirin was not additive. There was only one set of experiments in which there was no inhibition of glucose-stimulated insulin release after aspirin exposure. This occurred when islets were treated with aspirin during the pre-incubation period and rinsed before being placed in the experimental medium containing 300 mg% glucose alone. It may be concluded from these findings that aspirin exerts its effect maximally in the low glucose pre-incubation medium and that it inhibits the response of the islets to a high glucose stimulation with or without another exposure to aspirin.

The results of the present experiments using aspirin contradict the work of Chen and Robertson[24] who were able to restore the acute insulin response to hyperglycemic diabetics using 40 mg/min IV sodium salicylate. This was the only other study on aspirin and insulin release known to date. However, since their experiment also reported increased rates of glucose absorption, it contradicts other experiments on glucose absorption as well. Arvantikas[25] and co-workers found that aspirin inhibits glucose absorption by fifty per cent in humans given two to six grams of aspirin. Although Syvalahti[26] found no change in blood glucose levels of humans two hours after aspirin treatment, the dosage of aspirin (1 gram) may have been too low to show any effect. In his review of the "Clinical Pharmacology of Acetylsalicyclic Acid," Cohen[27] stated

that aspirin produced hyperglycemia and glycosuria in normal animals. He believed that the actions of aspirin included decreasing the aerobic metabolization of glucose while enhancing glucose-6-phosphate activity. These results are in agreement with the decreased insulin response caused by aspirin treatment since decreased plasma insulin would also alter the rate of glucose absorption from the gut if the experiments were performed in vivo.

It was expected that aspirin would have restored the ability of islets to respond to glucose stimulation or even enhanced that response. However, since the aspirin experiments show a significant inhibition of glucose-stimulated insulin release, it is suggested that aspirin acts by a different mechanism than simple inhibition of the formation of prostaglandins. While the aspirin is inhibiting the production of prostaglandins within the islet cells, there may also be additional receptor sites on the islets which will allow the aspirin to exert an inhibitory action of its own. The exact mechanism of aspirin on islet cells is unknown at the present time. It would be beneficial to correlate the effect of aspirin on the levels of cyclic AMP in the islets with the decreased insulin response. The involvement of cAMP as a second messenger in many other physiological systems leads to the possibility that cAMP may be involved in the modulation of insulin release by aspirin. However, the relationship of aspirin to cAMP levels in the islet cells remains to be established.

From this set of experiments, then, it can be seen that prostaglandins E_2 and U-51,095 inhibit insulin release from glucose-

stimulated islets but have no effect on non-stimulated islets. PGE_2 has no effect on the primary release of insulin. Aspirin, although a proven prostaglandin synthetase inhibitor, will also inhibit the response of islets to high glucose stimulation but be ineffective at low glucose levels. Finally, the glucose level at which the islets are maximally stimulated occurred at 600 mg% glucose in KRB. Incorporation of this data with work on cAMP will significantly clarify the role of the prostaglandins and aspirin on insulin release.

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